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(54) Title: PROCESS FOR THE STABILIZATION OF AN ACIDIC AQUEOUS PHASE COMPRISING CEPHRADINE

(57) Abstract: The invention relates to a process for the stabilization of an acidic aqueous phase comprising cephadrine, HCl, water and residual dichloromethane in solution, wherein the aqueous phase is subjected to a vacuum distillation. The invention also relates to a process for the production of cephadrine, wherein the acidic aqueous phase obtained after the separation of an organic phase and an aqueous phase produced in a chemical process for the production of cephadrine and which comprises cephadrine, HCl, water and residual dichloromethane in solution, is subjected to a vacuum distillation.

PROCESS FOR THE STABILIZATION  
OF AN ACIDIC AQUEOUS PHASE COMPRISING CEPHRADINE

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The invention relates to a process for the stabilization of an acidic aqueous phase comprising cephadine, HCl, water and dichloromethane in solution. The invention also relates to the stabilization of an acidic aqueous phase comprising cephadine, HCl, water and dichloromethane in solution, obtained in a chemical 10 process for the production of cephadine.

In a chemical process for the production of cephadine, the amino-group in 7-aminodesacetoxy cephalosporanic acid (7-ADCA) is acylated with a dihydrophenyl glycine side chain while dichloromethane is used as solvent. For example, D(-)- $\alpha$ -2,5-dihydrophenylglycine methyl sodium Dane salt and pivaloyl 15 chloride in dichloromethane may be added to a solution of 7-ADCA and 1,8-diazabicyclo-(5,4,0)-undec-7-ene (DBU) in dichloromethane. The resulting reaction mixture comprises cephadine with protected groups. The use of protected intermediates, such as cephadine with protecting groups, is characteristic for a chemical process for the production of cephalosporins, for example cephadine. To 20 remove the protecting groups, water and HCl are added to the reaction mixture. The reaction mixture and added acidic solution form a two phase system, which is subsequently separated into two separate phases, an organic phase comprising mainly dichloromethane and pivalic acid with some residual water and an aqueous phase comprising water, cephadine and HCl in solution, as well as residual dichloromethane. 25 Typically, the pH of the acidic aqueous solution, obtained through said chemical process for the production of cephadine and subsequent separation of the reaction mixture, is below 3.2. Subsequently, the formed cephadine is recovered from the aqueous phase by a pH shift; a complexing agent may be added. For example DMF or quinoline can be added to produce a solid cephadine complex, which is subsequently 30 converted to cephadine.

A problem is that, especially on an industrial scale, uncontrolled crystallization of cephadine as the HCl salt can spontaneously occur in an acidic aqueous phase comprising cephadine, HCl, water and residual dichloromethane in solution. By

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uncontrolled crystallization is meant (in this context); crystallization that occurs at a time without a deliberate external stimulus for example a pH shift). Uncontrolled crystallization of cephadrine as the HCl salt in a chemical process for the production of cephadrine reduces both the yield and quality of the product cephadrine. This is due to 5 the need for filtration of any prematurely crystallized cephadrine from the aqueous solution prior to the crystallization step. Filtration prolongs the overall product recovery and therefore leads to more degradation of the cephadrine product and therefore to a lesser quality of the product (among others a reduction in shelf-life).

EP 433 428 discloses a process in which a cephadrine salt is 10 stabilized in an acidic aqueous phase by addition of dimethylformamide (DMF) to the reaction mixture comprising a cephadrine salt and water. For the recovery of the cephadrine product, subsequent to the addition of DMF, the aqueous phase is heated and a base is added to increase the pH. Upon addition of the base, crystallisation of cephadrine.DMF solvate starts to occur. After dissolving the cephadrine.DMF solvate in 15 aqueous hydrochloric acid and adjusting the pH with a base, cephadrine is obtained.

However, in this method to prevent the crystallization of cephadrine as the HCl salt in an acidic aqueous phase, large amounts of DMF are needed. Large amounts of DMF are not advantageous, because this leads to the presence of residual DMF in the final product. Also, the use of these large amounts of DMF, makes this 20 process less efficient for the recovery of DBU.

It is the object of the invention to provide an improved process for the stabilization of an acidic aqueous phase comprising cephadrine, HCl, water and residual dichloromethane in solution. It is another object of the invention to provide a process for the production of cephadrine, in which the chance that uncontrolled 25 crystallization of cephadrine as the HCl salt occurs is minimized. It is another object of the invention to provide a process for the production of cephadrine in which the chance that uncontrolled crystallization of cephadrine as the HCl salt occurs is minimized and in which the use of DMF is avoided. It is another object of the invention to provide a process for the production of cephadrine in which the chance that uncontrolled 30 crystallization of cephadrine.HCl occurs is minimized and in which the use of DMF is avoided and in which DBU can be more efficiently recovered.

It has surprisingly been found that by subjecting an acidic aqueous phase, comprising cephadrine, HCl, water and residual dichloromethane in solution, to

a vacuum distillation, a stable aqueous phase is obtained in which the chance that uncontrolled crystallization of cephadine as the HCl salt occurs, is minimized. An added advantage of the invention is that the quality of cephadine is improved with respect to containing less dichloromethane in the final product.

5                 The term 'residual dichloromethane' means that there is more than 1.0% w/w dichloromethane present in the acidic aqueous phase. Typically, the content of residual dichloromethane in the acidic aqueous phase obtained after the separation of an organic phase and an aqueous phase produced in a chemical process for the production of cephadine is in the range of 3 to 7% w/w.

10                 The invention therefore relates to a process for the stabilization of an acidic aqueous phase comprising cephadine, HCl, water and residual dichloromethane in solution, characterized in that the aqueous phase is subjected to a vacuum distillation. The invention can suitably be applied in a chemical process for the production of cephadine, wherein after the separation of an organic phase and an aqueous phase, an acidic aqueous phase comprising cephadine, HCl, water and residual dichloromethane in solution is produced. Therefore, the invention also relates to a process for the production of cephadine, in which the acidic aqueous phase obtained after the separation of an organic phase and an aqueous phase produced in a chemical process for the production of cephadine and which comprises cephadine, HCl, water and residual dichloromethane in solution, is subjected to a vacuum distillation. Thereby, a process has been found, in which an acidic aqueous phase comprising cephadine, HCl, water and residual dichloromethane in solution is stabilized without the use of DMF and wherein the chance that uncontrolled crystallization of cephadine as the HCl salt occurs is minimized. This has the advantage that cephadine can be obtained in a higher purity and therefore, less isolation and subsequent purification steps, for example recrystallization steps, are needed.

15                 Cephadine may be prepared in a chemical process comprising: (i) acylating the amino-group in 7-aminodesacetoxy cephalosporanic acid (7-ADCA) with a dihydrophenyl glycine side chain in the presence of dichloromethane, resulting in a reaction mixture; (ii) adding HCl and water to the reaction mixture, resulting in a two phase system, said two phase system comprising an organic phase and an acidic aqueous phase, said acidic aqueous phase comprising water, cephadine, HCl and

dichloromethane. The acidic aqueous phase and organic phase may be separated in any suitable way, for instance phase separation. Preferably, said acylating is carried out in the presence of pivaloyl chloride and/or 1,8-diazabicyclo-(5,4,0)-undec-7-ene.

Preferably, the process comprises acylating said 7-aminodesacetoxy cephalosporanic

- 5 acid using D(-)- $\alpha$ -2,5-dihydrophenylglycine methyl sodium Dane salt. In a preferred embodiment, the process comprises adding D(-)- $\alpha$ -2,5-dihydrophenylglycine methyl sodium Dane salt and pivaloyl chloride in dichloromethane to a solution of 7-ADCA and 1,8-diazabicyclo-(5,4,0)-undec-7-ene (DBU) in dichloromethane.

Preferably, the pH of the acidic aqueous phase is below 3.2.

- 10 The vacuum distillation is preferably carried out as a continuous distillation. The temperature and the pressure of the vacuum distillation may vary between wide ranges. Preferably, the vacuum distillation in the process according to the invention is carried out at a temperature between 0 and 40°C, preferably between 10 and 40°C, more preferably between 30 and 40°C, most preferably between 34 and 15 38°C. Preferably, the vacuum distillation is started at a temperature between 34-38°C. Preferably, the vacuum distillation is carried out at a pressure below 450 mm Hg. At the start of the vacuum distillation, the pressure is preferably between 450 and 350 mm Hg.

- 20 The acidic aqueous phase to be subjected to the vacuum distillation comprises dichloromethane, for instance more than 1.0% w/w dichloromethane, for instance between 3 to 7% w/w dichloromethane.

The vacuum distillation may be carried out in any suitable way, such as to separate dichloromethane from the acidic aqueous phase. As a result the concentration of dichloromethane in the acidic aqueous phase is decreased.

- 25 Surprisingly, the separation of dichloromethane from an acidic aqueous phase comprising cephradine, HCl, water and dichloromethane is found to result in an increased stability of the acidic aqueous solution. Accordingly, a process is also provided comprising separating dichloromethane from an acidic aqueous phase, said acidic aqueous phase comprising cephradine, HCl, water and dichloromethane. The 30 concentration dichloromethane in the acidic aqueous phase prior to said separating may for instance be more than 1.0% w/w dichloromethane, for instance between 3 to 7% w/w dichloromethane. Preferably, the separation is carried out such as to obtain a concentration dichloromethane in the acidic aqueous phase of less than 1.0% w/w,

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preferably less than 0.8% w/w. The vacuum distillation is preferably continued until the quantity of dichloromethane remaining in the aqueous phase after vacuum distillation is less than 1.0% w/w, preferably less than 0.8 % w/w. Decreasing the concentration of dichloromethane below these values further improves the stability. Accordingly, the  
5 invention also provides an acidic aqueous solution comprising cephadrine, HCl, water and dichloromethane, the concentration dichloromethane in said acidic aqueous solution being less than 1.0% w/w, preferably less than 0.8% w/w. The amount of dichloromethane present in the aqueous phase can be analyzed with gas chromatography (GC). Alternatively, the end-point of the vacuum distillation can be  
10 determined by the pressure. The vacuum distillation is preferably continued until the pressure of the vacuum distillation is equal to or less than 150 mm Hg.

Cephadrine can be isolated from the stabilized acidic aqueous solution by changing the pH to a pH suitable for crystallization. The temperature at which this crystallization takes place is preferably between 40 and 70°C, more  
15 preferably between 48 and 62°C.

The invention is illustrated by way of the following examples.  
However, these examples are not meant to restrict the invention.

Examples  
20 Example I. Including the introduction of a vacuum distillation step resulting in a stable aqueous solution of cephadrine and hydrochloric acid.

1) MIXED ANHYDRIDE PREPARATION  
A suspension of N-methylacetamide (21.7 grs; 0.30 moles), D(-)- $\alpha$ -  
25 2,5-dihydrophenylglycine methyl sodium Dane salt (269.9 grs; 0.987 moles), gamma-picoline (0.055 ml; 0.6 mmoles) in dichloromethane (980 ml) was cooled to -20°C, treated with pivaloyl chloride (119.7 grs; 0.991 moles) at -10°C during 10 minutes, and cooled to -35°C.

This system was called preparation A  
30

2) 7-ADCA SOLUTION  
Dichloromethane (679 ml) was cooled to 5°C and 7 ADCA (175 grs; 0.817 moles) was loaded. Then diazabicyclo (5,4,0) undec-7-ene (140 grs; 0.920

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moles) was added with stirring to obtain complete solution.

This system was called preparation B

### 3) COUPLING (ACYLATION)

5 Preparation B was added into preparation A, keeping the temperature at -35°C. The resulting reaction mixture C was stirred for 3 hours.

### 4) HYDROLYSIS

10 Water (980 ml) and hydrochloric acid 35% (210 grs; 2.0 moles) were added to reaction mixture C and the temperature was brought up to 15°C. The pH was in the range of 1.5 to 2.0. Then, after stirring for 15 minutes, the layers were separated.

### 5) VACUUM DISTILLATION

15 The aqueous (upper) layer obtained in step 4 was immediately warmed up to 38°C and simultaneously distilled under vacuum conditions (initial pressure of about 360 mmHg; final pressure 150 mmHg) to remove the residual dichloromethane. The vacuum distillation was stopped when the GC (gas chromatography) showed a dichloromethane content of 0.7% w/w. A stable (with  
20 respect to uncontrolled crystallization of cephadrine hydrochloric salt), clear solution,  
containing cephadrine was obtained.

### 6) ISOLATION

The obtained solution was warmed up to 60 °C and cephadrine was  
25 crystallized by adding triethylamine until pH=5. After filtration, 220g (0.60 moles; 73% yield) of cephadrine (99% assay on dry basis) were obtained. The purity of the thus obtained cephadrine was within the specifications of the Chinese Pharmacopeia.

Comparative example A. Excluding a vacuum distillation step resulting in a potentially unstable aqueous solution of cephadrine and hydrochloric acid.

Steps 1-4 were performed according to example I. Step 5 was not performed and instead the aqueous (upper) layer obtained in step 4 was kept during 10 minutes at 15°C with stirring. After this period of time, a slurry of crystals was obtained.

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The crystals were filtered, washed first with 100 ml of isopropyl alcohol followed by 300 ml of n-hexane and dried. The product was identified as cephadrine as the hydrochloric acid salt.

CLAIMS

1. Process for the stabilization of an acidic aqueous phase comprising  
5 cephadrine, HCl, water and dichloromethane, said process comprising  
subjecting said acidic aqueous phase to a vacuum distillation.
2. Process for the production of cephadrine, wherein the acidic aqueous phase  
obtained after the separation of an organic phase and an aqueous phase  
produced in a chemical process for the production of cephadrine and which  
10 comprises cephadrine, HCl, water and dichloromethane, is subjected to a  
vacuum distillation.
3. Process according to claim 1 or 2, wherein the vacuum distillation is carried  
out as a continuous vacuum distillation.
4. Process according to any of claims 1-3, wherein the vacuum distillation is  
15 carried out at a temperature between 0 and 40 °C.
5. Process according to any of claims 1-4, wherein the vacuum distillation is  
started at a pressure between 450 and 350 mm Hg.
6. Process according to any one of claims 1-5, wherein the acidic aqueous  
phase comprises more than 1.0% w/w dichloromethane.
- 20 7. Process according to claim 6, wherein the acidic aqueous phase comprises  
between 3 to 7% w/w dichloromethane.
8. Process according to claim 6 or claim 7, wherein the vacuum distillation is  
continued until the quantity of dichloromethane remaining in the aqueous  
solution is less than 1.0% w/w.
- 25 9. Process according to claim 8, wherein the vacuum distillation is continued until  
the quantity of dichloromethane remaining in the aqueous solution is less than  
0.8 % w/w.
10. Process according to any of claims 1-9, wherein the vacuum distillation is  
continued until the pressure of the distillation is equal to or less than 150 mm  
30 Hg.
11. Process according to any of claims 1-10, wherein the stabilized acidic  
aqueous phase obtained by the process according to any of claims 1-9 is  
further subjected to an isolation step in which cephadrine is crystallized by

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- changing the pH to a pH where cephradine crystallizes.
12. Process according to claim 11, wherein the isolation step takes place at a temperature in the range from 48 to 62°C.
13. Acidic aqueous solution comprising, cephradine, HCl, water and dichloromethane, in which solution the concentration dichloromethane is less than 1.0% w/w, preferably less than 0.8% w/w.
- 5 14. Process comprising subjected the solution according to claim 13 to an isolation step in which cephradine is crystallized by changing the pH to a pH where cephradine crystallizes.
- 10 15. Process according to claim 14, wherein the isolation step takes place at a temperature in the range from 48 to 62°C.
16. Cephradine in crystalline form obtainable by the process according to any one of claims 11,12,14,15.

## INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 C07D501/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, BEILSTEIN Data, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91 00865 A (GEMA SA) 24 January 1991. (1991-01-24) cited in the application page 5, line 5 - line 25	16
A	US 3 912 726 A (DIASSI PATRICK ANDREW ET AL) 14 October 1975 (1975-10-14) example 2	1-15
X	US 3 912 726 A (DIASSI PATRICK ANDREW ET AL) 14 October 1975 (1975-10-14) example 2	16
A	US 3 912 726 A (DIASSI PATRICK ANDREW ET AL) 14 October 1975 (1975-10-14) example 2	1-15

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Patent family members are listed in annex.

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## INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9100865	A	24-01-1991	AT 112780 T DE 69013305 D1 DE 69013305 T2 DK 433428 T3 WO 9100865 A1 EP 0433428 A1 ES 2023560 A6 ES 2063974 T3 JP 8009630 B JP 4500819 T US 5278157 A	15-10-1994 17-11-1994 18-05-1995 05-12-1994 24-01-1991 26-06-1991 16-01-1992 16-01-1995 31-01-1996 13-02-1992 11-01-1994
US 3912726	A	14-10-1975	AR 201027 A1 JP 49116094 A	06-02-1975 06-11-1974

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